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Phenotypic and Pathogenic Characterization of Leaf Fungi of Yam *(Dioscorea spp)* Varieties Grown In Côte D'Ivoire

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A B S T R A C T

Yam (*Dioscorea spp*) occupies an important place in the diet of the populations of Côte d'Ivoire. It is a real source of starch and therefore generates enormous commercial potential. However, the decrease in production due to pest attacks represents a real threat to this crop. This study was conducted with the aim of improving yam production in Côte d'Ivoire. To do so, isolations carried out on yam leaves showing symptoms of foliar diseases have allowed us to identify 9 fungal genera. These were *Colletotrichum sp., Fusarium sp., Pestalotiopsis sp., Pestalotia sp., Botryodiplodia sp., Aspergillus sp., Mucor sp., Curvularia sp.* and *Phytophtora sp.* Among these fungi, the genus *Colletotrichum sp.* was the most isolated with a rate of 56% followed by the *Fusarium* and *Pestalotia* genera (8%). Pathogenicity tests performed on healthy leaves of two yam varieties revealed that the *Dioscorea alata* is more susceptible to fungi compared to *Dioscorea rotundata.* The largest average diameter of necrosis was caused by *Pestalotiopsis sp.* (5.97 cm) on the *Dioscorea alata* variety while the smallest was caused by *Colletotrichum sp.*9 on *Dioscorea rotundata* (0.5 cm). Combatting these fungi need to be developed for effective management of leaf diseases of yam in Côte d'Ivoire.

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Introduction

In tropical regions, root and tuber crops (cassava, sweet potato, potato and yam) are important food crops. Among these root and tuber crops, yam (*Dioscorea spp.*), with a global production of 75 million tons in 2021, is the staple diet for more than 500 million people in selected tropical countries in Africa, the Caribbean, Oceania, and Latin America (Onyeka et al., 2006; FAOSTAT, 2021**)**. In West Africa, where the large share of production is carried out, yam is one of the main sources of starch that actively contributes to the food security of the populations (Dansi et al., 2013).

In Côte d'Ivoire, yam occupies an important place in food crop production, with a yield of 7.8 million tons per year, which ranks the country as the second largest yam producing country after Nigeria (FAOSTAT, 2021). This production is mainly based on the species, *Dioscorea cayenensis-rotundata* and *Dioscorea alata*, which are grown on a large scale (Dansi et al.,2013). It is consumed by more than two-thirds of the population in several forms (Digbeu et al., 2009). Apart from the tuber, yam leaves are also used in traditional African pharmacopoeia for the treatment of various health problems (Kouakou, 2004). However, yam production is limited by enormous biotic and abiotic constraints including parasitic attacks such as viruses (Séka et al., 2009; Toualy et al., 2014), nematodes (Anjorin et al., 2014), bacteria (Denis, 2005) and fungi responsible for leaf necrosis (Amusa et al., 1996). Fungal leaf diseases are a real threat to this crop. Among these diseases, anthracnose is the most frequent fungal disease in Côte d'Ivoire and has an impact on yam production as it causes yield losses that can reach more than 90% (Green et Simon, 2007). The aim of this study is to analyze the diversity of fungal pathogens associated with yam leaf symptoms for an effective control approach.

Materials and Methods

Study Site

Samples were collected in localities located in the major yam-producing areas of eastern, northern, central and south-western Côte d'Ivoire (Figure 1). The characteristics of these different localities are shown in Table 1.

Plant Material

Leaves with characteristic fungal disease symptoms were used for fungal isolation and healthy leaves were used for pathogenicity testing of the different fungi isolated. The plant material used is composed of two lots of yam leaves (*Dioscorea alata*) and (*Dioscorea cayenensis-retoundata*). These two yam species were selected for pathogenicity testing after the surveys, taking into account the varieties grown and their susceptibility to foliar diseases.

Surveys and Sample Collection

Surveys were carried out on yam plots at the vegetative stage, 3 to 6 months after planting. Leaves showing different symptoms likes necrosis, spots and burns of fungal diseases observed on the different yam varieties were described, collected, photographed and then classified in envelopes. The samples were then taken to the laboratory for isolation.

Isolation of Fungi Associated with Symptoms

Isolation of fungal strains was done from explants (leaf fragments) taken from leaves showing symptoms of fungal diseases. Symptoms due to fungal attack were identified with reference to studies by (Yao et al., 2017). These explants were collected at the margin of the symptoms and cultured on Potato Dextrose Agar (PDA) medium.

Preparation of the PDA Medium

For the preparation of 1 liter of PDA medium, 20 g of mashed potato, 20 g of glucose and 20 g of agar-agar were weighed on a balance and put in a jar. The volume of the mixture was adjusted to 1 liter by adding distilled water. This medium was autoclaved at 121°C for 30 minutes under a pressure of 1bar. The resulting medium was dispensed into 9 cm diameter petri dishes under a laminar flow hood in the presence of a flame (Camara, 2011; Yao et al., 2017).

Seeding of Explants

Explants were washed with tap water, spread on sterile blotting paper (to remove excess water) and disinfected with 90° alcohol (Camara, 2011). Using sterile forceps, leaf fragments of about 1 cm^2 were cut at the growing front of the symptom and then seeded into petri dishes containing solidified PDA culture medium, with four explants per Petri dish. Two petri dishes were used per symptom. Petri dishes were labeled, sealed, and then incubated for 3 to 4 days at laboratory room temperature (25 ± 2°C).

Purification of Mushrooms

The fungal colonies observed underwent a first purification step from the Petri dish used for seeding the explants. The fungi were individually transplanted on new PDA media. Purification of the fungal strains consisted of transplanting each strain several times onto new media under sterile conditions so that a pure, individualized colony could be isolated (Camara, 2011).

Once the pure fungal strains had been obtained, the cultural characteristics were described in terms of the coloration, appearance and growth pattern of the mycelial colonies. Colony explants were mounted between slide and coverslip and observed under a light microscope at magnification (G × 400). Organs such as mycelia and spores were characterized. The Botton et al. (1990) identification key was used for strain identification.

Frequency of fungi isolation

The isolation frequencies of fungi were calculated according to the formula of Walder (1996):

$$\mathrm{FI}\ (\%) = \frac{\mathrm{NI}}{\mathrm{NTI}} \times 100$$

FI (%): Frequency of isolation in percent

NI: Number of isolations of a fungal genus in all samples

NTI: Total number of isolations of all fungal genera.

Table 1. Characteristics of yam production areas surveyed in Côte d'Ivoire

Areas	Localities	Types of Soil	Pluviometry (mm)	Temperature (°C)	Climate
East	Bondoukou Bouna Tanda	deep sandy-clay	1400-2500	24-29	Topical wet and dry
North	Dabakala; Katiola Kanawolo	ferruginous	1150-1350	26-30	Sec tropical
Center	Bouaké Tiébissou Lolobo Djebonoua	ferrallitic ferruginous	1000-1700	25-38	Topical humide
South-West	Buyo Soubré Gagnoa Agboville	ferrallitic	1300-1600	26-30	Sub-equatorial

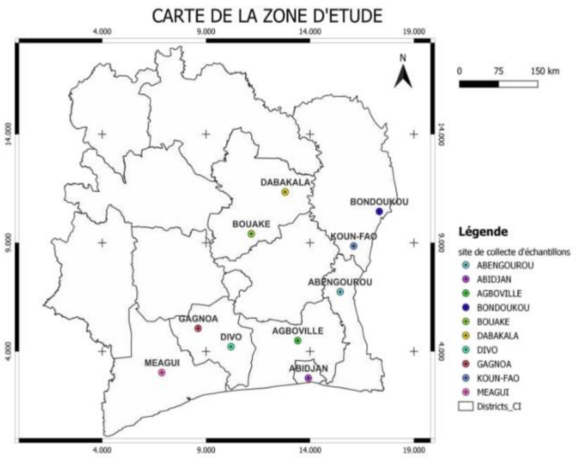


Figure 1. Map of Côte d'Ivoire showing sample collection areas

Spores Quantification and Growth Measurement of Colletotrichum Strains

To obtain sporal suspensions, five mycelial washers were placed in a test tube containing 10 ml sterile distilled water. The spore suspension was homogenized by vortexing, filtered through filter paper and the quantity of conidia per milliliter was assessed per strain under a light microscope using a Malassez cell. Three counts were made for each strain.

To determine the radial growth of the mycelium, daily measurements were taken from two perpendicular lines drawn on the underside of each Petri dish. Average diameters per day were obtained by applying the following formula (Hmouni et al., 2005):

$$\mathrm{Dm} = \frac{\mathrm{D1} + \mathrm{D2}}{2}$$

Dm: average daily growth diameter
D1: growth diameter along axis 1
D2: growth diameter along axis 2

Pathogenicity Test

The pathogenicity test was performed with 11 strains of *Colletotrichum sp.*; 2 strains of *Fusarium sp.* and *Pestalotia sp.* and one strain for the genera *Pestalotiopsis sp.*; *Botryodiplodia sp.*; *Curvularia sp.*; *Mucor sp.* and *Aspergillus sp.*

Healthy leaves of two yam varieties, Bètè Bètè (*Dioscorea alata*) and Assawa (*Dioscorea rotundata*) located between positions 4 and 7 from the stem apex were used for this test. These leaves were collected from three- to four-month-old plants. Three leaves were used per fungus and per variety. Three additional leaves per variety representing the controls were also used.

The leaves were first rinsed with tap water under a hood and placed on sterile blotting paper and disinfected with 90° alcohol. They were then placed in each 90 mm diameter Petri dish containing blotting paper cut into slices and moistened with sterile distilled water. The 12-day-old fungal colonies were used for inoculation (Silué et al., 2018). With sterile punches, mycelial discs of 5 mm diameter were formed. Referring to the work of Touré (2014), inoculations consisted of the deposition of a mycelial disc in the center of the upper surface of the leaf (Figure 2). The blotting papers were moistened with distilled water, every other day if necessary to maintain relative humidity and facilitate inoculum growth. Observations were made daily after inoculation for fourteen days.

Description of Symptoms

At the end of the experiment, the symptoms that appeared on the inoculated leaves were described. The description concerned the shape, contour and color of the symptoms (Yao et al., 2017).

Average Prevalences

To assess the prevalence of the symptom on each leaf, the number of leaves showing necrosis symptoms during the experiment was counted for each fungus and for each species. The percentage of the average prevalence was calculated according to the following formula (Yao et al., 2017).

$$\mathrm{PM}(\%) = \frac{\sum \mathrm{NFM}}{\mathrm{NTFM}} \times 100$$

PM (%): Average prevalence of disease,
NFM: Number of diseased leaves,
NTFM: Total number of diseased leaves,

Average Severity

The average severity of necrosis was assessed. Estimates were made using the Urban and Lebeda (2004) rating scale. For each yam leaf observed, the severity of necrosis was noted.

0: No symptoms on the leaves,
1: less than 25% of the leaf area infected,
2: 26-50% of leaf area infected,
3: 51-75% of leaf area infected,
4: more than 75% of the leaf area infected).

$$\mathrm{SI}\ (\%) = \frac{\sum(\mathrm{xi} \times \mathrm{ni})}{\mathrm{Z} \times \mathrm{N}} \times 100$$

SI (%): Severity index.
xi: Individual note to the disease symptom on each leaf.
ni: Number of times the rating score.
Z: Number of inoculated leaves.
N : Highest score on the scale.

Average Diameters of Necrosis

At the end of the experiment, the diameters of the necrosis induced by the different fungal strains were measured with a graduated ruler along two perpendicular axes drawn on the lid of the petri dish (Yao et al., 2017). The average diameter was evaluated 14 days after inoculation.

***Re-isolation* (koch's postulate)**

In order to verify the responsibility of fungal strains in the induction of necrosis, isolations were made from the observed symptoms. The fungal strains that infected the leaves were isolated from the induced necrosis. Koch's postulate was thus verified.

Statistical Analysis

The data obtained were subjected to an analysis of variance (ANOVA) with Statistica 7.1 software. In case of significant difference between the means, Newman Keul's test at the 5% threshold was used to classify the homogeneous groups.

Results

Observed Symptoms of Leaf Diseases

Various symptoms were observed on the leaves of all yam varieties in the surveyed localities (Figure 3). Three types of symptoms were observed : necrosis, spots and burns.

Isolation and Morphological Characterization of Fungi

Ten fungal genera were isolated from yam leaves showing symptoms of leaf diseases. Among them nine were identified. These were *Colletotrichum sp, Fusarium sp, Pestalotiopsis sp, Pestalotia sp, Botryodiplodia sp, Aspergillus sp, Mucor sp, Curvularia sp and Phytophtora sp* (Figure 3). The greatest species diversity was observed in the fungus *Colletotrichum sp.*

Relationship between Symptomatic Forms and Isolated Fungal Genera

Several fungal genera have been associated with a single type of symptom, just as a single fungus has been responsible for several symptoms. The *Colletotrichum* genus has been associated with several symptoms such as black necroses with yellow halo, brown spots, yellow necroses interspersed with black spots, marginal black and brown necroses, brown necroses with yellow patch, brown necroses with yellow halo, black spots with yellow halo, black necroses without halo, black and brown scorch marks, marginal brown scorch marks and scattered black spots on the leaf with blackening of the veins.

The genus *Fusarium* was isolated from deformed leaves showing black spots and yellow necroses dotted with black spots. The fungus *Pestalotioptis sp.* was associated with black and white necroses. *Botryodiplodia sp.* was associated with brown necrosis with a yellow halo. The fungus *Phytophtora sp.* was isolated from brown necroses with a yellow patch. The *Pestalotia sp.* genus has been isolated from three different symptoms. These are black spots with yellow halo, large black spots and small brown spots with yellow halo. The genera *Aspergillus*, *Mucor* and *Curvularia* were isolated respectively from brown discoloration, burns with yellow halo and burns without halo.

Isolates of Colletotrichum sp.

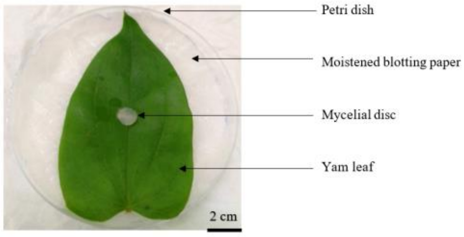
Eleven different strains of *Colletotrichum sp.* were isolated (Figure 4 A à K). The color of the thallus varied from white to black, yellow and gray. Some strains showed a pink or brown thallus mixed with white, not visible on the reverse side of the Petri dish. The thallus was abundant or contuncous in most cases, but also short in some cases. Microscopic examination revealed a wide variety of conidial shapes. Spores were cylindrical, oval, round with rounded or pointed ends. Conidia of various sizes were also observed in other strains. All strains of this genus showed branched mycelia under the light microscope, with the presence of septa for *Colletotrichum* sp. 1, 2, 5 and 6.Growth diameters at day 7 on PDA culture medium vared according to strain. It was maximal for the *Colletotrichum sp.* 7 strain, which reached an average growth diameter of 85 on day 7. he smallest mean growth diameter value (51.5 mm) was obtained with the *Colletotrichum sp.*9 strain. Conidia were predominantly cylindrical (92.85%) and round (7.14%). Spores quantities varied from strain to strain. *Colletotrichum sp.*2 recorded the highest number of spores per milliliter (26,10^6), while *Colletotrichum sp.*11 (3.2, 10^6 spores /ml) was the least sporulating strain (Table 2).

Isolates of Fusarium sp.

Two different strains of *Fusarium sp* were isolated :

The thallus of the first strain is yellow-white on the upper surface of the Petri dish and orange-yellow on the underside. Its cultural appearance is cottony. Microscopically, the conidia observed are elongated and curved with a branched, septate mycelium (Figure 4 L).

The thallus of the second isolate is white on the upper surface of the Petri dish and light yellow on the reverse. The cultural appearance is cottony, high in the center and short at the ends of the Petri dish. Under the light microscope, the conidia observed are curved, some with pointed, multi-partitioned ends (Figure 4 M). The mycelium is branched but not septate.



Figures 2. Yam leaf inoculated with a PDA disc (control)

Isolates of Pestalotia sp.

Two different isolates of *Pestalotia sp* were isolated.

The thallus of the *Pestalotia sp* 1 has a white coloration on the upper surface of the petri dish. Black sclerotia appear on the middle surface. The color is light yellow on the reverse side. The sclerotia are much more visible on the reverse side than on the top of the petri dish, but at a low density. The cultural aspect is lined and short. Microscopically the conidia are clavate, light to dark brown in the middle part and hyaline at the ends with three appendages. They have a tapered, transparent tip with dark medial segments and septate mycelium (Figure 4N).

The thallus of the *Pestalotia sp* 2 is yellow-white on the upper surface of the petri dish. In culture black sclerotia appear on the surface of the medium. The color is yellow on the reverse side. The sclerotia appearing on the reverse side have a low density. The cultural aspect is lined, short and radiating. Microscopically the conidia are clavate, dark brown in the middle part with two appendages. They have a tapered, transparent tip, medial segments years are darker (Figure 4O).

Isolates of Pestalotioptis sp.

The appearance of the mycelium is lined and short. In the culture white balls appear. Under the microscope the conidia are clavate, dark brown in the middle and without appendages. They have a tapered, transparent tip and the medial segments are more so mber (Figure 4P).

Isolates of Botryodiplodia sp.

Its cultural aspect is cottony, lined, high. In the culture black balls appear. Microscopic observation gives large oval conidia with rounded ends, surrounded by a sheath in the central part. The black septum is oriented in the direction of the smallest diameter of the conidia (Figure 4Q).

Table 2. Morphometric characteristics of isolated *Colletotrichum sp*. strains

Strains	Growth diameter at 7th day (mm)	Average growth speed (mm/day)	Appearance of the aerial thallus	Average number of conidia per milliliter ($\times 10^6$)
Colletotrichum sp.1	65,50c	8,19c	Cottony	8,0e
Colletotrichum sp.2	73,50b	9,19b	Abundant	26,0a
Colletotrichum sp.3	75,50b	9,44b	Hyalin	13,2c
Colletotrichum sp.4	66,83c	8,35c	Cottony	10,0d
Colletotrichum sp.5	61,27d	7,66d	Cottony	11,2c
Colletotrichum sp.6	81,67a	10,21a	Cottony	17,6b
Colletotrichum sp.7	85,00a	10,63a	Cottony	3,6g
Colletotrichum sp.8	59,17d	7,40d	Fibrous	4,0f
Colletotrichum sp.9	51,50f	6,44f	Cottony	13,6c
Colletotrichum sp.10	61,00d	7,63d	Cottony	10,4d
Colletotrichum sp.11	73,17b	9,15b	Cottony	3,2g

In the same column, values bearing the same letters are statistically identical at the 5% threshold according to the Newmann-Keuls test.

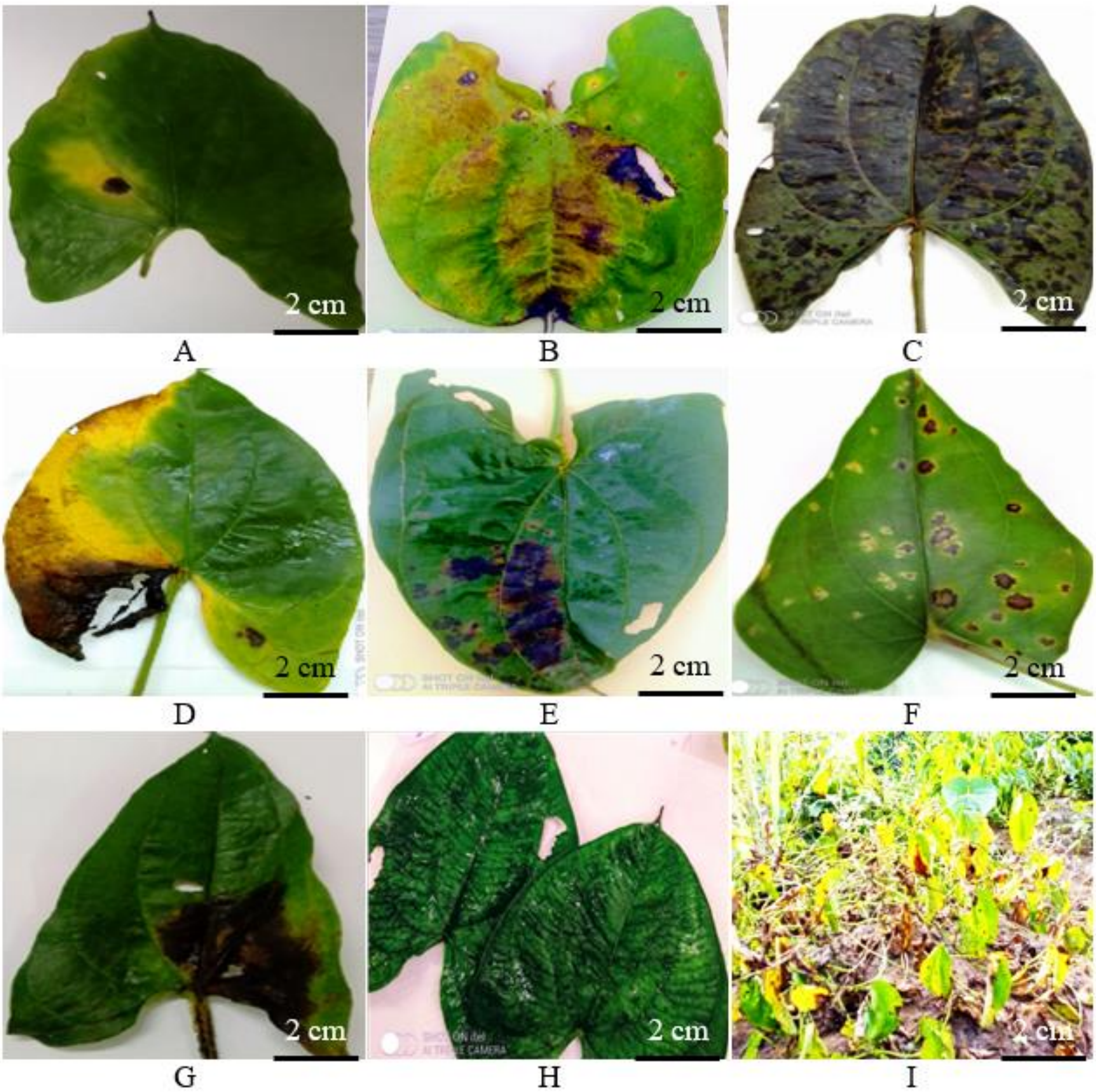


Figure 3. Some leaf disease symptoms observed on the leaves of different yam varieties

A : Brown necrosis with yellow patch; B : Black and brown burns; C : Large black spots all over the leaf; D : Brown and yellow marginal necrosis; E : Black burns; F : Brown necrosis with yellow halo; G: Black and brown necrosis with blackening of veins; H : Black spots + leaf deformations; I : Whole plant wilting.

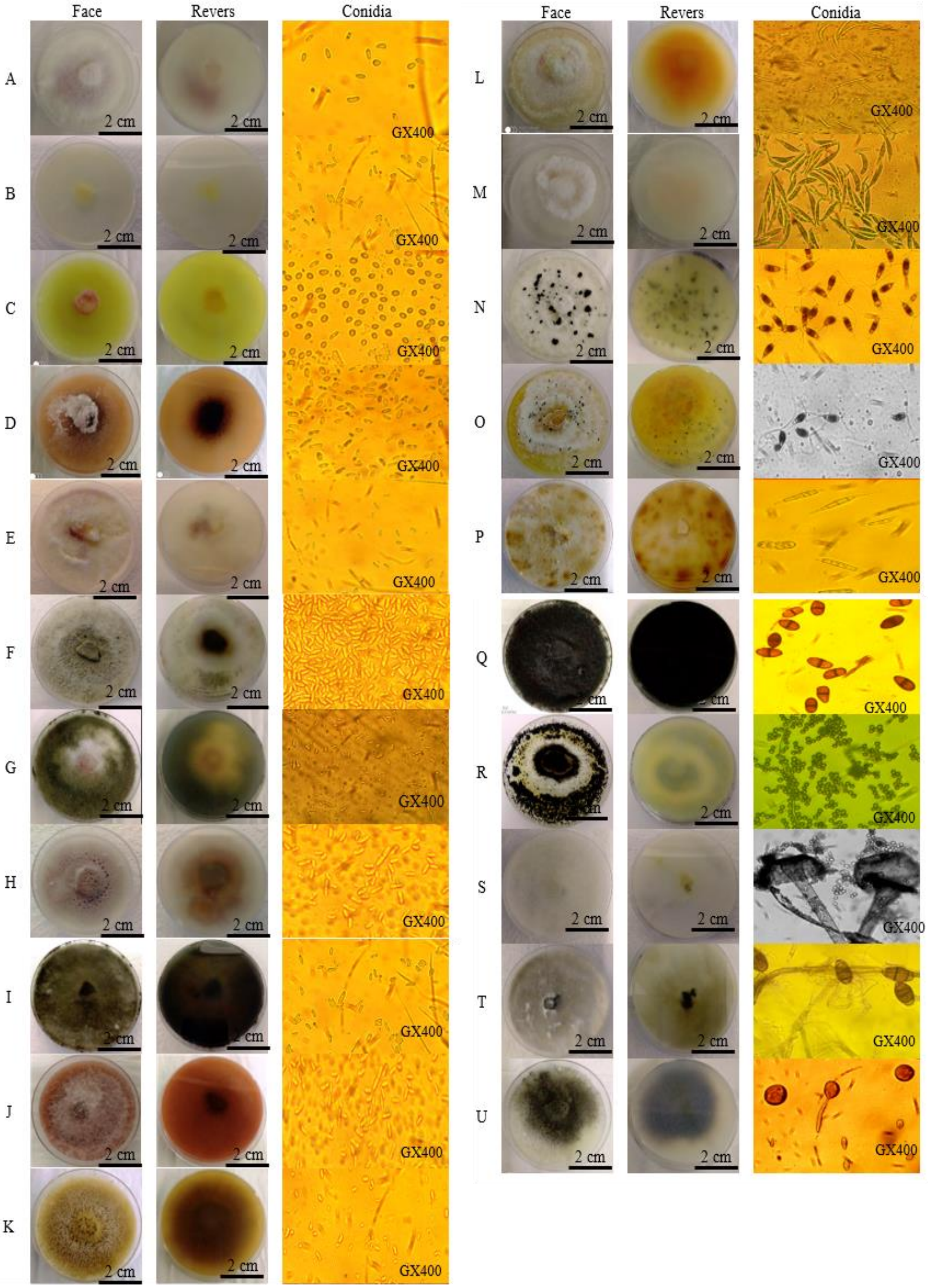


Figure 4. Fungi isolated from yam leaves showing symptoms of foliar diseases
A; *Colletotrichum* sp. 1; B: *Colletotrichum* sp. 2; C: *Colletotrichum* sp. 3; D: *Colletotrichum* sp. 4; E: *Colletotrichum* sp. 5; F: *Colletotrichum* sp. 6, G *Colletotrichum sp 7;* H: *Colletotrichum sp 8;* I: *Colletotrichum sp 9;* J: *Colletotrichum sp 10;* K: *Colletotrichum sp 11;* L: *Fusarium sp. 1;* M: *Fusarium sp. 2;* N: *Pestalotia sp. 1.;* O: *Pestalotia sp. 2 ;* P: *Pestalotioptis sp. ;* Q: *Botryodiplodia sp. ;* R: *Aspergillus sp;* S: *Mucor sp;* T: *Curvularia sp;* U: *Phytophthora sp*

Isolates of Aspergillus sp.

The thallus of the mushroom is white-yellow on the upper side of the petri dish and yellow-brown on the reverse side. The cultural aspect is lined, short, with an irregular growth. Microscopic observation yields circular, tip-attached spores (Figure 4R).

Isolates of Mucor sp.

The thallus of the mushroom has a grayish coloration of face and yellow-grayish on the reverse. The cultural aspect is abundant, high, uniformly growing. Microscopic observation shows oval, grayish-brown conidia. The mycelium is branched (figure 4S).

Isolates of Curvularia sp.

The thallus of the mushroom is of color gray of face and yellow-gray to the reverse. The cultural appearance is lined and concentrically crescent. Microscopically the conidia are curved, penta-septate s, alternating gray and black (Figure 4T).

Isolates of Phytophtora sp

The thallus of the fungus is colored in white-verses of face and white-violet on the reverse. Its cultural appearance is cottony, elevated, growing and irregular. Microscopically the conidia are oval and elongated on one side (Figure 4U).

Frequencies of isolation of fungi

Figure 4 shows the isolation frequencies of the fungi. The genus *Colletotrichum sp* was isolated most frequently with a frequency of 56% **(**Figure 5). It is followed by the genus *Pestalotiopsis sp* and *Fusarium sp* with a frequency of 8%. All other fungi had the same frequency of 4%.

Symptoms Caused by the Fungi after Inoculation

All the fungi inoculated on yam leaves belonging to the *Dioscorea alata* and *Dioscorea rotundata* species induced different types of symptoms compared with the control (Figures 6 and 7), with the exception of the *Colletotrichum sp. 10* and *Botryodiplodia sp*. strains (Figure 8). These two strains did not induce any symptoms on yam leaves of the Assawa variety (*Dioscorea rotundata*). These included brown, black, brown with yellow halo and brown without yellow halo necroses on yam leaves of the Bètè Bètè variety (*Dioscorea alata*), and brown, brown with yellow halo and brown without yellow halo necroses on those of the Assawa variety (*Dioscorea rotundata)*. Symptoms were not specific to a single fungal genus inoculated. Several fungal genera were responsible for a single type of symptom. Thus, brown necrosis with yellow halo was induced by the genera *Colletotrichum Pestalotia* and *Botryodiplodia*. The genera *Aspergillus, Mucor* and *Pestalotiopsis* caused black necrosis. Brown necrosis without halo was caused by the *Curvularia* and *Fusarium* genera.

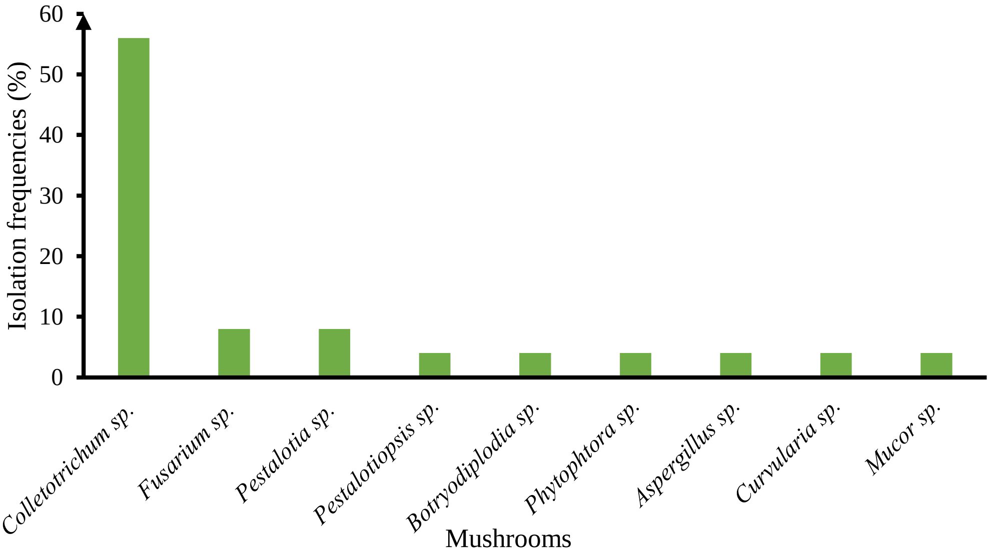


Figure 5. Isolation frequencies of different fungal genera isolated from yam leaves collected in Côte d'Ivoire.

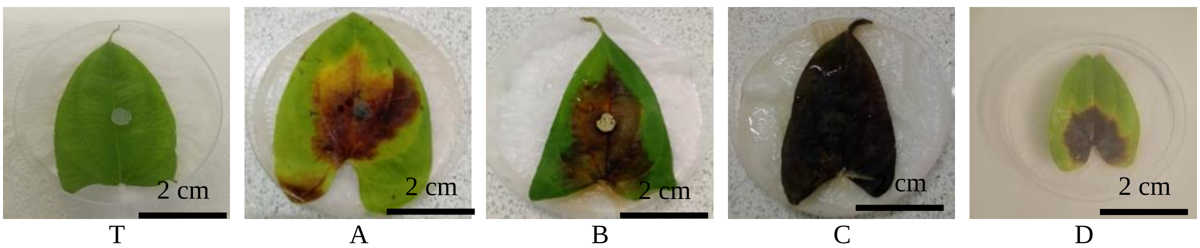
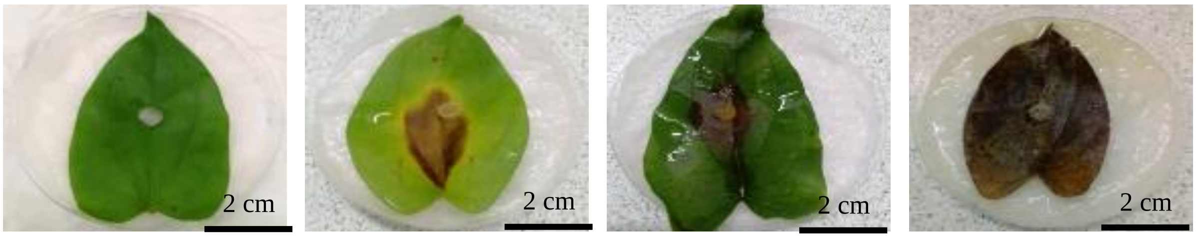


Figure 1. Different types of necrosis induced in vitro by fungal genera deposited on leaves of the Bètè Bètè variety (*Dioscorea alata*) 14 days after inoculation.

A: Brown necrosis with yellow halo ; B: Brown necrosis without yellow halo; C: Black necrosis ; D: Brown necrosis with yellow halo; T: Control leaf



T A B C

Figure 7. Different types of necrosis induced in vitro by fungi deposited on leaves of the Assawa variety *(Dioscorea rotundata*) 14 days after inoculation.

A: Brown necrosis with yellow halo B : Brown necrosis without halo; C : Black necrosis; T : Control leaf

A B

Figure 2. Fungi that did not induce any symptoms after inoculation on leaves of the Assawa variety (Dioscorea rotundata)

A: *Colletotrichum* sp. 10 ; B: *Botryodiplodia* sp.

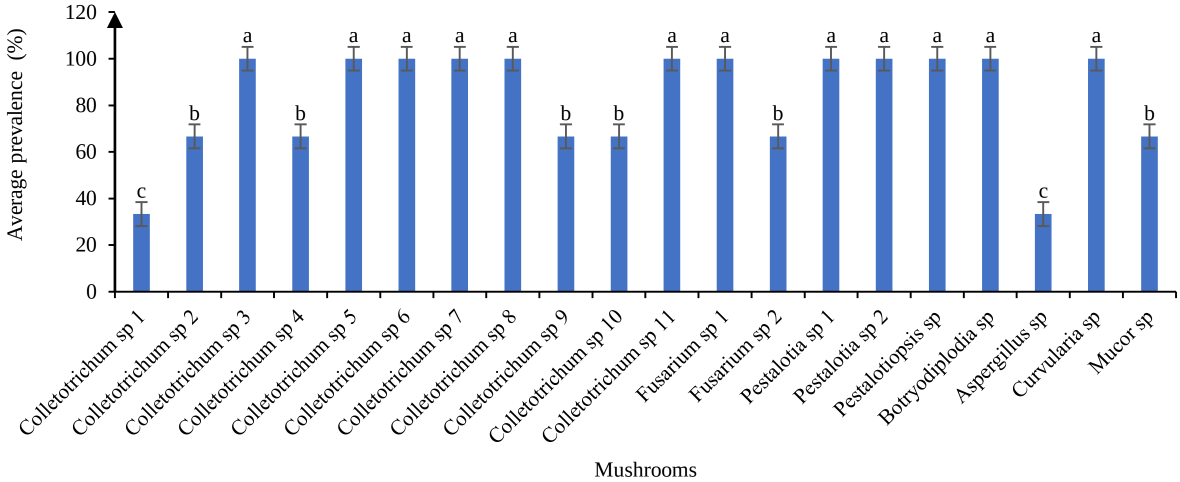


Figure 9. Average prevalences of necrosis obtained 14 days after inoculation of the fungi on the leaves of *Dioscorea. alata.*

Bars with the same letters are statistically identical at the 5% threshold according to the Newmann-Keuls test.

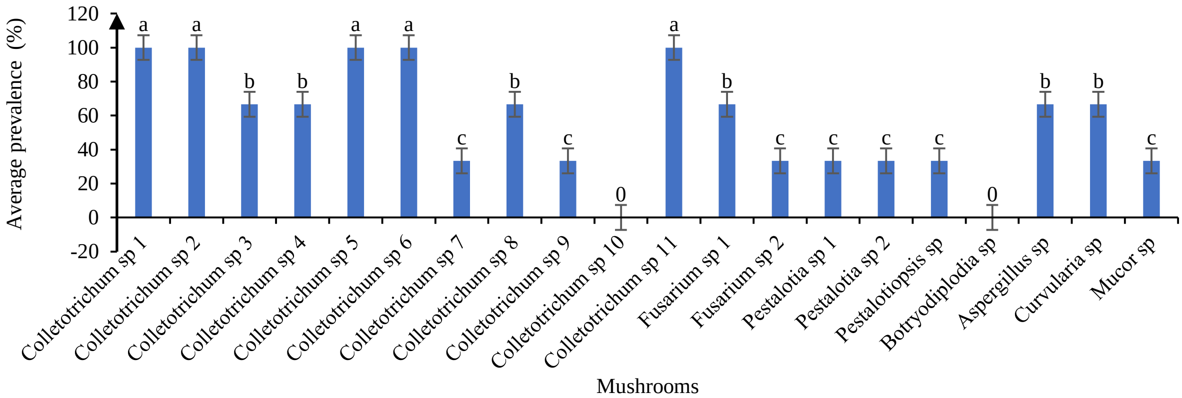


Figure 10. Average prevalences of necrosis obtained 14 days after fungus inoculation on leaves of *Dioscorea. rotundata* species.

Bars with the same letters are statistically identical at the 5% threshold according to the Newmann-Keuls test.

Average Prevalences of Necroses

The average prevalences of necrosis varied according to species and fungi inoculated. The average prevalence of necrosis in *Dioscorea alata* specie ranged from 33.33 to 100%. Rates of 100% were obtained for *Colletotrichum sp.* 3, *Colletotrichum sp* 5, *Colletotrichum sp.* 6, *Colletotrichum sp.* 7, *Colletotrichum sp.* 8, Colletotrichum *sp* 11, *Fusarium sp.* 1 *Pestalotia sp.* 1, *Pestalotia sp.* 2, *Pestalotiopsis sp*, *Botryodiplodia sp*, and *Curvularia sp. Colletotrichum sp*. 2, *Colletotrichum sp*. 4 *Colletotrichum sp*. 9, *Colletotrichum sp.* 10, *Fusarium sp.* 2, and *Mucor sp* induced 66.66%. Finally *Colletotrichum sp1* and *Aspergillus sp., on the other hand,* induced a rate of 33.33%. (Figure 9).

In contrast, the average prevalence of necrosis in *Dioscorea rotundata* species ranged from 0 to 100%. The rates of 100% were obtained with *Colletotrichum* sp.1, *Colletotrichum sp*.2, *Colletotrichum* sp.5, *Colletotrichum sp*.6 and *Colletotrichum* sp.11. *Colletotrichum sp*.3, *Colletotrichum* sp.4, *Colletotrichum* sp.8, *Fusarium* sp.1, *Aspergillus sp* and *Curvularia sp* induced a rate of 66.66%. *Colletotrichum* sp 7, *Colletotrichum sp* 9, *Fusarium sp* 2, *Pestalotia sp.* 1, *Pestalotia* sp. 2, *Pestalotiopsis* sp, and *Mucor sp* induced 33.33%. Finally the fungi *Colletotrichum* sp.10 and *Botryodiplodia sp.* did not induce symptoms. (Figure 10). The results obtained show that the *Dioscorea alata* variety is more susceptible to fungal attacks than the *Dioscorea rotundata* specie.

Average severity of necrosis

The average necrosis severities varied according to the species and the fungi inoculated. The average necrosis severity of the specie *Dioscorea alata* varied from 0 to 100%. The fungi *Fusarium sp* .1, *Pestalotia sp.* 1 and *Botryodiplodia sp* caused the most severe symptoms to 100%. The other fungi had an impact on the leaves, except for the fungus *Fusarium sp.* 2 which did not induce any symptoms on the leaves. (Figure 11). The average necrosis severity of the *Dioscorea rotundata* specie ranged from 0 to 91.66%. *Colletotrichum sp.* 6 and *Colletotrichum sp.* 11 fungi caused the most severe symptoms at 91.66% compared to the control. The other fungi had an impact on leaves, except *Colletotrichum sp*. 7, *Colletotrichum sp*. 9, *Colletotrichum sp.* 10 and *Botryodiplodia sp* induced no symptoms on leaves. (Figure 12). Statistical analysis showed that there was a significant difference between the fungi inoculated in relation to the two yam species used. The fungi *Colletotrichum sp.* 3,6 and 11 ; *Fusarium* sp.1 and *Botryodiplodia sp.* were the most virulent on *Dioscorea alata*, while *Colletotrichum* sp.6 and 11 were the most virulent on *Dioscorea rotundata*.

Average diameters of Necrosis

Average necrosis diameters varied with yam varieties and fungi inoculated. The average diameters of fungus-induced necrosis on the leaves of *Dioscorea alata* species ranged from 0.92 to 5.97 cm. The largest average diameter was obtained with *Pestalotia sp.* 1 (5.97 cm). *Fusarium sp.* 2 induced the smallest mean diameter of necrosis (0.92 cm). Statistical analyses showed a significant difference between the means of necrosis. Eight homogeneity groups were obtained (Table 3).

The average diameters of fungus-induced necrosis on the leaves of the *Dioscorea rotundata* specie ranged from 0.00 to 5.25 cm. The largest average diameter was induced by *Colletotrichum sp*. 6 (5.25 cm). In contrast, *Colletotrichum sp*. 10 and *Botryodiplodia sp. did* not induce any necrosis on the leaves of this variety. Statistical analyses showed a significant difference between the means of necrosis. Eight homogeneity groups were obtained. Statistical analyses showed a significant difference ($P<0.000612$) between the mean necrosis diameters of *Dioscorea alata* and *Dioscorea rotundata* species.

Re-isolation of Fungal Pathogens

Isolations made from the different necroses observed resulted in the same fungi being inoculated. Koch's postulate is thus verified.

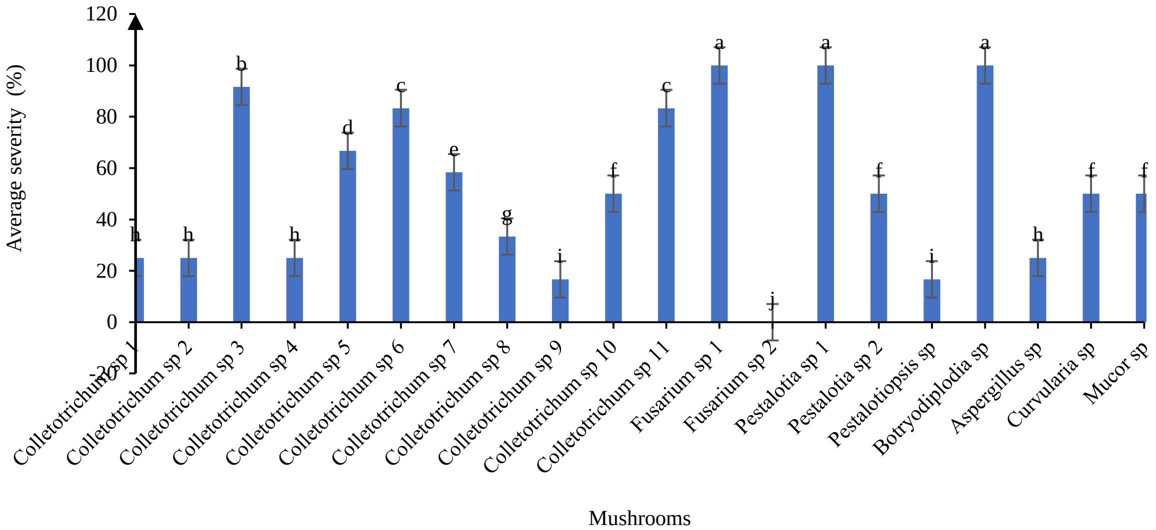


Figure 11. Average severity of necrosis obtained 14 days after inoculation of on Dioscorea alata leaves.
Bars with the same letters are statistically identical at the 5% threshold according to the Newmann-Keuls test.

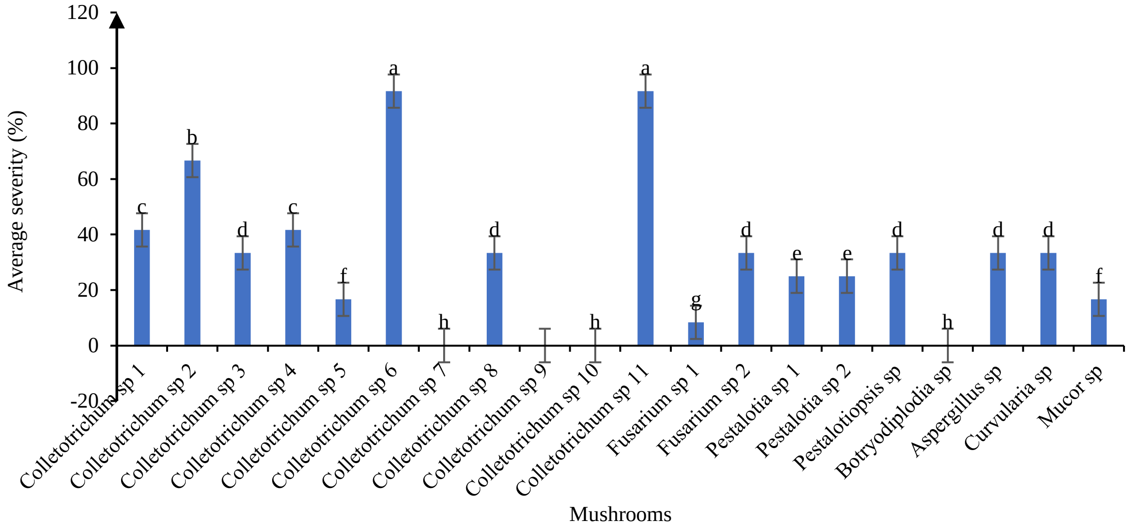


Figure 12. Average severity of necrosis observed 14 days after inoculation of fungi on the leaves of *Dioscorea rotundata*

Bars with the same letters are statistically identical at the 5% threshold according to the Newmann-Keuls test.

Table 3. Average diameters of necrosis induced by fungi inoculated on leaves of *Dioscorea alata and Dioscorea rotundata*

Mushrooms	Average diameter of necrosis (cm)	
	Dioscorea alata	*Dioscorea rotundata*
Colletotrichum sp. 1	1,55 ± 0,25d	4,35 ± 0,81b
Colletotrichum sp. 2	2,58 ± 0,72c	4,47 ± 0,64b
Colletotrichum sp . 3	5.62 ± 0.3a	2.85 ± 1.6c
Colletotrichum sp . 4	2.40 ± 1.35c	2.83 ± 1.55c
Colletotrichum sp . 5	4,07 ± 1,23bc	2,50 ± 0,78c
Colletotrichum sp . 6	5.07 ± 0.63ab	5.25 ± 1.01a
Colletotrichum sp . 7	3.87 ± 1.19c	0.62 ± 0.61e
Colletotrichum sp . 8	3.87 ± 1.16 c	2.02 ± 1.56cd
Colletotrichum sp . 9	2,08 ± 1,4cd	0,50 ± 0,5e
Colletotrichum sp . 10	3,58 ± 1,88c	0,0 ± 0,0e
Colletotrichum sp. 11	3.93 ± 1.2c	4.98 ± 1b
Fusarium sp . 1	5,28 ± 0,25a	1,68 ± 2,3d
Fusarium sp . 2	0,92 ± 0,53e	2,37 ± 2,36c
Pestalotia sp . 1	5,97 ± 1,25a	0,83 ± 0,5e
Pestalotia sp. 2	5.17 ± 0.72a	0.83 ± 0.83e
Pestalotiopsis sp	1.95 ± 0.51d	1.88 ± 1.88d
Botryodiplodia sp	4,72 ± 0,83b	0,00 ± 0,0e
Aspergillus sp	1,27 ± 1,26d	2,57 ± 1,38c
Curvularia sp	4.32 ± 1.1 b	2,25 ± 0,5c
Mucor sp	3,02 ± 0,1c	1.63 ± 1.31d
Probability (p)	P =0,000612	

In the same column, values bearing the same letters are statistically identical at the 5% threshold according to the Newmann-Keuls test.

Discussion

The Diagnosis of foliar symptoms in the production zones surveyed revealed a number of fungal disease symptoms on the yam species *Dioscorea alata* and *Dioscorea cayenensis-rotundata*. These include black, brown, grey and yellow spots, necroses and burns. These results corroborate those of Amusa et al. (1996) whose work in Nigeria on the leaves of *Dioscorea alata* and *Dioscorea rotundata* showed the presence of three types of necrosis from which the fungi were isolated. Similar results were obtained by Touré (2014) during work carried out on the diagnosis of leaf symptoms in four localities in Côte d'Ivoire. The same type of necrosis was observed on the leaves of different varieties of *Dioscorea alata* and *Dioscorea rotundata* species. This similarity in necrosis type could be the presence or attack of the same fungal genus on yam leaves. Studies by Amusa et al. (1996) showed that the main causal agent of anthracnose on *Dioscorea alata* and *Dioscorea rotundata* leaves was *Colletotrichum gloeosporioides*. Different types of symptoms were observed on the leaves of the same yam variety. This diversity of symptoms could be due to the presence of several fungal genera parasitizing the leaves of the same yam variety. Studies carried out in Ghana showed that two types of necrosis were observed only on necrotic leaves of *Dioscorea alata* (Twumasi, 1986). In addition, studies carried out by Touré (2014) in four localities in Côte d'Ivoire, specifically Nassian, Soubré, Babadougou and Toumodi on *Dioscorea alata* leaves showed the presence of three different types of necrosis on the Bètè Bètè variety.

Several fungal genera were isolated, nine of which were identified. These were *Colletotrichum sp. ; Fusarium sp. ; Pestalotia sp. ; Pestalotioptis sp. ; Botryodiplodia sp. ; Aspergillus sp. ; Mucor sp.* and *Curvularia sp*. These results indicate that these different fungal genera are associated with the symptoms observed on yam leaves collected in production zones. Work carried out on the symptomatology, etiology and incidence of yam leaf disease has highlighted the presence of fungi such as : *Aspergillus sp.; Botryodiplodia sp.; Colletotrichum sp.; Curvularia sp.; Fusarium sp.; Mucor sp.; Pestalotiopsis sp.* (Touré, 2014). The fungal diversity associated with necrotic yam leaves has been confirmed by the work of Achar et al. (2013). These authors isolated *Colletotrichum gloesporioides, Fusarium oxysporum, Pestolatia macrotrichia, Cercospora* and *Cladosporium* fungi from *Dioscorea alata* and *Dioscorea bulbifera* leaves. Isolation frequency was highest for the *Colletotrichum* genus. This fungal genus is widely recognized as responsible for anthracnose on the leaves and fruit of many plants (Freeman, 1998 ; Arauz, 2000). Studies by several authors have shown the preponderance of this fungal genus on yam leaves. Indeed, the work of Touré (2014) in Côte d'Ivoire isolated the *Colletotrichum* genus at 28.9% on yam leaves. Furthermore, the high presence of the *Colletotrichum* genus among the fungi isolated from *Dioscorea alata* leaves was confirmed by the work of Abang et al., (2003) during their study. The work of several authors has shown that this fungal genus is responsible for anthracnose in several crops around the world. Indeed, Silué (2019) isolated this pathogen from various cashew organs with a rate of 63.45%. Also, Ehui et al. (2019) had demonstrated that a very high diversity of this fungus is associated with cassava anthracnose in Côte d'Ivoire. The majority of inoculated fungi infected the leaves of both yam species. The fungi were more virulent on the Bètè Bètè variety of the *Dioscorea alata* species compared with the Assawa variety (*Dioscorea rotundata*). These results corroborate those of Kwasi et al. (2019) whose work in Togo shows that varieties of the species *Dioscorea alata* are highly susceptible to inoculation by strains of *Colletotrichum sp.* Studies carried out by Touré (2014) in Côte d'Ivoire on *Dioscorea alata* leaves showed that the fungi *Colletotrichum sp., Rhizoctonia sp, Rhizoctonia sp., Botryodiplodia sp., Pestalotiopsis sp.* and *Curvularia sp.* were able to induce necrosis on detached leaves. Different types of necrosis have been induced by in vitro inoculation of detached *Dioscorea alata* and *Dioscorea rotundata* leaves with the fungi used. Studies carried out by Amusa et al. (1996) on the leaves of *Dioscorea alata* and *Dioscorea rotundata* showed three types of necrosis induced after inoculation with the fungi. All the symptoms induced by these various inoculated fungi, as well as those observed during field sampling, are generally attributed to anthracnose. This confusion is due to the fact that anthracnose caused by fungi of the *Colletotrichum* genus is the most widely described leaf disease of yam in the literature. However, this study has enabled us to identify the fungi associated with each type of symptom observed. Thus, the genera *Colletotrichum* and *Pestalotia* have been associated with several symptoms. *Colletotrichum* was isolated from symptoms such as black necrosis with yellow halo, brown spots, yellow necrosis with black dots, black and brown marginal necrosis, brown necrosis with yellow patch, brown necroses with yellow halo, black spots with yellow halo, black necroses without halo, black and brown burns, marginal brown burns and scattered black spots on the leaf with blackening of the veins. *Pestalotia* fungi were associated with three symptoms : black spots with yellow halo, large black spots and small brown spots with yellow halo. This result is contrary to that of Mignouna et al. (2001) who attributed these same types of symptoms to yam anthracnose. Touré (2014) isolated the *Colletotrichum* genus from *Dioscorea alata* leaves showing the large black spots.

The fungus *Botryodiplodia sp*. was associated with brown necroses with a yellow halo. This result disagrees with that of Touré (2014), who isolated *Curcularia sp.* from the same type of necrosis in Côte d'Ivoire. Furthermore, brown necrosis with a yellow halo had been described as that caused by *Curcularia sp*. in Guadeloupe (INRA, 2005).

The genus *Fusarium* has been isolated from deformed leaves showing black spots and yellow necroses dotted with black spots. The fungus *Pestalotioptis sp*. was associated with black and white necroses. The genera *Aspergillus, Mucor* and *Curvularia* have been isolated respectively associated with brown discoloration, burns with yellow halo and burns without halo. However, all these symptoms were described as that of yam anthracnose in the work of Yao et al., (2017) in Côte d'Ivoire. These symptoms have also been observed by several authors on yam leaves and seedlings as characteristic symptoms of yam anthracnose around the world, particularly in the Pacific**,** Guadeloupe and Nigeria (Amusa et al., 2003 ; Jacqua et al., 2008 ; Bakayoko et al., 2022). In addition to anthracnose caused by fungi of the *Colletotrichum* genus, this study highlights the diversity of leaf diseases of yam in Côte d'Ivoire. The prevalences and severities obtained after the inoculations depended on the fungi and yam varieties. The fungi *Colletotrichum sp.* 3,6 and 11 ; *Fusarium* 1 and *Botryodiplodia sp.* were the most virulent on *Dioscorea alata*, while *Colletotrichum sp*.6 and 11 were the most virulent on *Dioscorea rotundata*. Studies carried out in Côte d'Ivoire on the leaves of *Dioscorea alata* and *Dioscorea cayenensis-rotundata* species showed a difference in the prevalences and severities obtained following the pathogenicity test (Touré, 2014). The work of Yao et al, (2017) also showed a diversity of necroses caused by different fungi on yam leaves. The average diameters of necroses obtained on leaves of *Dioscorea alata* and *Dioscorea rotundata* species varied during the experiment according to variety and fungus. The largest average diameter for the Bètè Bètè variety belonging to the *Dioscorea alata* species was obtained with the *Pestalotia sp.* 1 fungus. For the Assawa variety of the *Dioscorea rotundata* species, the largest diameter was obtained with the fungus *Colletotrichum sp*. 6. These results show that necrosis size varies according to the degree of aggressiveness of the fungal strains. Inoculations carried out by Yao et al., (2017) on *Dioscorea alata* leaves with mycelial discs of several fungal genera showed different necrosis sizes and by Rekad (2018) during studies conducted at Algérie on Tomato leaves inoculated with isolates of *Phytophthora infestans*.

Conclusion

This study highlighted the diversity of fungal genera associated with yam leaf disease symptoms in Côte d'Ivoire. Among these fungi, the *Colletotrichum* genus was the most isolated. These fungal genera isolated from diseased yam leaves proved effectively pathogenic on detached feulae of both yam varieties. The fungi *Colletotrichum sp.* 3,6 and 11 ; *Fusarium sp.* 1 and *Botryodiplodia sp* were the most virulent on *Dioscorea alata*, while *Colletotrichum sp*.6 and 11 were the most virulent on *Dioscorea rotundata*. These fungi need to be effectively controlled in order to stem yield losses due to foliar diseases and preserve yam orchards in Côte d'Ivoire and around the world.

Competing Interests

The authors declare that they have no competing interests in the publication of this work.

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